

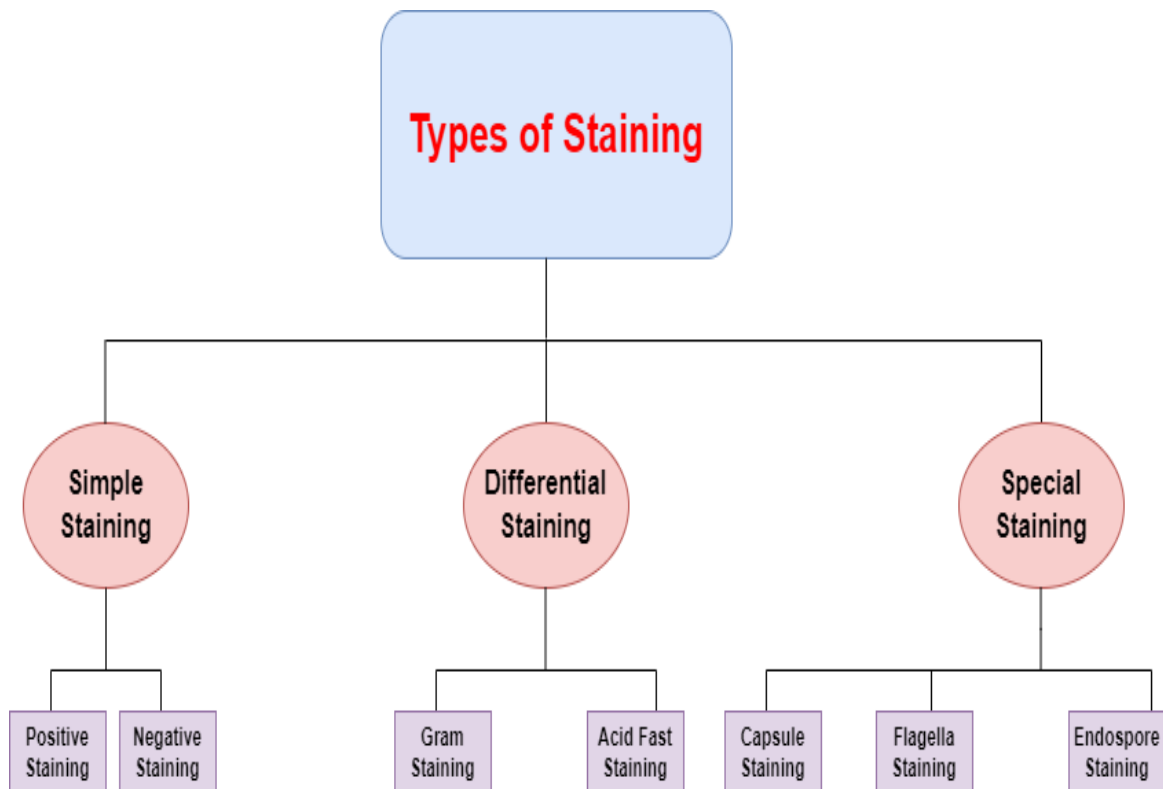
## Lab6 : Staining

Staining is an Important process in microbiology. To study all structure and shape of microorganisms one must know and able to see the microorganism, and for that, it has to be colored by utilizing simple, monochrome, negative, differential staining and other staining techniques.

❖ Stain divided into

**1 - Simple stain**

**2 - Differential stain**



**Differential stain** These staining procedures are used to distinguish organisms based on staining properties. They are slightly more elaborate than simple staining techniques that the cells may be exposed to more than one dye or stain, for instance use of Gram staining which divides bacteria into two classes-Gram negative and Gram positive.

### ➤ Preparation and Fixation of Bacteria for Staining

#### ❖ The Importance of Fixation

- 1 – Cell adhesion to the slide
- 2- Killed the stabilized cell
- 3 – Coagulation of protein substance

### Fixation Methods

#### 1 – Heat fixation

The slide passing on the Bunsen burner several times, bearing in mind that using heat in an exaggerated manner will distort the shape and composition of the cell.



#### 2 – Chemically fixation

The slide fixed by using methyl alcohol 95% for one minute.

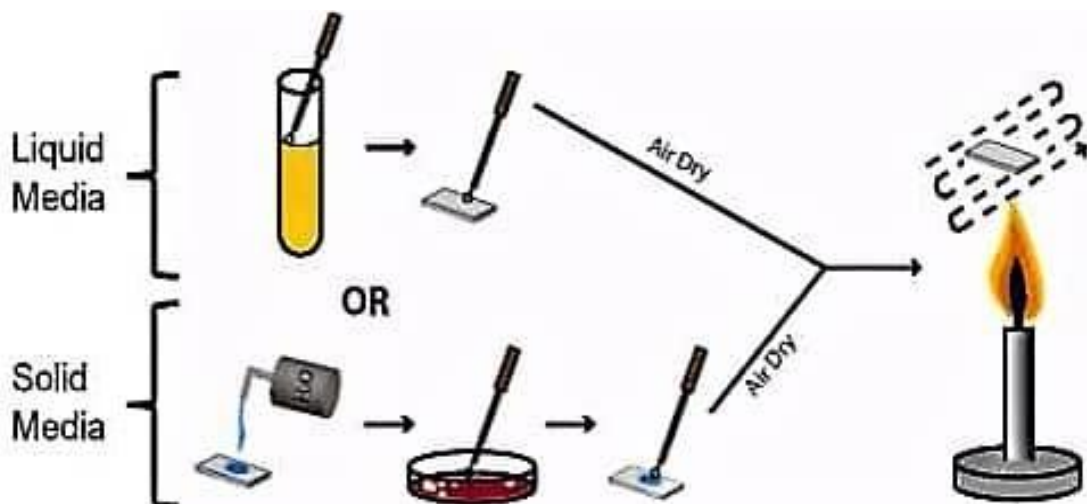


## ❖ Gram stain

Gram stain or Gram staining, also called Gram's method, is a method of staining used to distinguish and classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria. The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique. Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. **Gram-positive** cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet. **Gram-negative** cells have a thinner peptidoglycan layer that allows the crystal violet to wash out on addition of ethanol. They are stained pink or red by the counterstain, commonly safranin or fuchsine.

### Materials Needed:

- ❖ Glass slides
- ❖ Bacterial culture
- ❖ Crystal violet (primary stain)
- ❖ Iodine solution (mordant)
- ❖ 95% ethanol or acetone (decolorizer)
- ❖ Safranin (counterstain)
- ❖ Water
- ❖ Bunsen burner (for heat-fixing)
- ❖ Microscope



## Procedure:

### 1. Prepare the bacterial smear:

- ❖ Place a drop of water on a clean glass slide.
- ❖ Using a sterilized inoculating loop, take a small amount of bacterial culture and mix it with the drop of water.
- ❖ Spread the mixture thinly across the slide to create a smear.
- ❖ Allow the smear to air dry completely.

### 2. Heat-fix the smear:

- ❖ Pass the slide quickly through the flame of a Bunsen burner 2-3 times, with the smear side facing up. This kills the bacteria and fixes them to the slide.

### 3. Apply the Crystal Violet (primary stain):

- ❖ Cover the smear with crystal violet stain and let it sit for **60 seconds**.
- ❖ Rinse the slide gently with distilled water.

### 4. Apply Iodine (mordant):

- ❖ Cover the smear with iodine solution and let it sit for **60 seconds**. This helps fix the crystal violet dye to the bacterial cell walls.
- ❖ Rinse the slide gently with distilled water.

### 5. Decolorization (using ethanol or acetone):

- ❖ Flood the smear with 95% ethanol or acetone for **10-20 seconds** (until no more purple color washes off). This step differentiates Gram-positive from Gram-negative bacteria.
- ❖ Rinse the slide immediately with distilled water to stop the decolorization.

### 6. Apply Safranin (counterstain):

- ❖ Cover the smear with safranin for **30-60 seconds**. This stains the Gram-negative bacteria pink or red.
- ❖ Rinse the slide gently with distilled water.

### 7. Dry the slide:

- ❖ Gently blot the slide dry with bibulous paper (or allow it to air dry).

### 8. Examine under the microscope:

- ❖ Place the slide under a microscope and observe using oil immersion at 100x magnification.

## Results:

- **Gram-positive bacteria:** Appear **purple** or **blue** due to the retention of the crystal violet-iodine complex in their thick peptidoglycan layer.
- **Gram-negative bacteria:** Appear **pink** or **red** because their thinner cell walls allow the crystal violet to be washed away, and they take up the safranin counterstain.

## Gram Stain

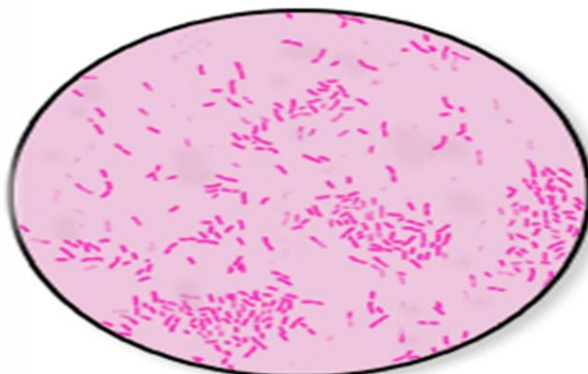
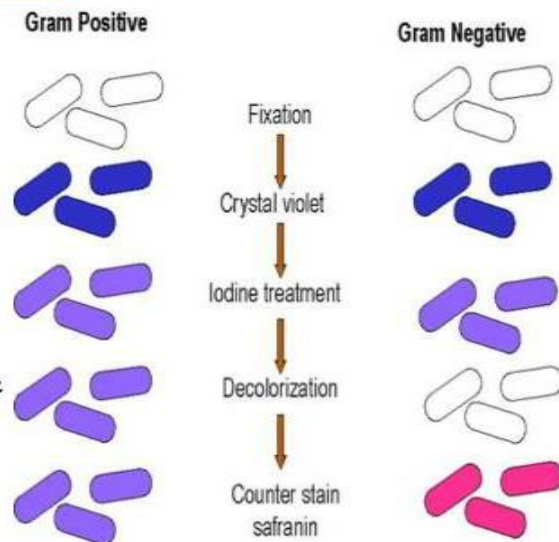
### Principle of staining technique:

Primary stain:- Crystal Violet

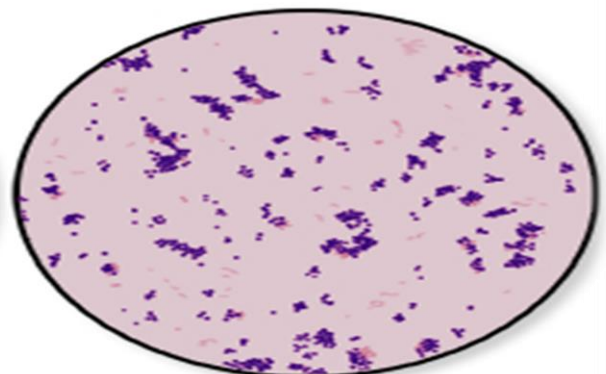
Mordant (fixes the dye):- Iodine

Decolorizing agent:- Alcohol/Acetone

Counter stain:- Safranin



Gram-negative



Gram-positive